The Development of Aplysia punctata.

By

A. M. Carr Saunders and Margaret Poole.

With Plate 22 and 20 Text-figures.

This work was begun by one of us in the spring of 1909, at the Zoological Station at Naples, when holding the Oxford biological scholarship.[1] Owing to various reasons, the chief of which was ill-health, little more was done there than to collect material. It has been completed with assistance in reconstruction of sections and illustrations at Oxford in the department of Comparative Anatomy during the winter 1909–10. We must here express our gratitude to Prof. Bourne for the opportunity he has afforded us, and the encouragement he has given us to complete the work.

The bionomics of Aplysia have been described with great care by Carazzi and Mazzarelli. The former deals at length with the deposition of the eggs and their early development, while the latter, in his monograph on Aplysia (**14**), describes the general bionomics of the genus. The three common species found at Naples are punctata, limacina, and depilans. Carazzi (**5**), in his work on the cell-lineage of Aplysia, made observations on all three, and found little difference between them. Our results refer entirely to Aplysia punctata.

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[1] I wish to take this opportunity of expressing my thanks to the staff at Naples for their continual kindness during the time I was there, and especially to Professor Meyer and Professor Eisig for their valuable advice with regard to methods.—A.M.C.S.

A number of stages of A. limacina were also examined, but the difference is insignificant.

Carazzi states that Aplysia punctata disappears in May to reappear again in the winter. We were able, however, to obtain this species in large numbers until the middle of June. No difficulty was experienced in keeping Aplysia in the aquarium, and they laid eggs in great quantities. The eggs develop normally, and equally well if kept in jars or in the tanks with circulation, provided only that the water be changed every two days or so. Early in the summer the eggs were at times attacked by bacteria, but if enough spawn was kept it was always possible to have some at the stage required in a healthy condition. Later in the year the eggs were attacked by algæ, and the embryos destroyed long before the free-swimming stage was reached. This was a more serious trouble than the bacteria, but the difficulty can be avoided by keeping the spawn in filtered water in the dark, where the algæ do not develop. The rate of development varies with the temperature of the water. This is described by Carazzi for the different species. In April some fifteen days elapsed between the deposition of the eggs of A. punctata and the emergence of the free-swimming larvæ from the capsules. It is possible to keep the larvæ in jars for some time, but even though they be kept in circulating water, they always die within a short time without exhibiting any change of structure. Mazzarelli states that he kept some larvæ of Bulla striata alive for twenty days, which is far longer than we ever succeeded in keeping Aplysia larvæ, but even these showed no change during that period. No one has yet raised any Opisthobranch larvæ through the metamorphosis, and there is therefore a large gap in our knowledge of the embryology of the group, for not only in the free-swimming larva are certain adult organs, such as the heart and pericardium and the gonads and genital ducts, entirely undeveloped, but the interpretation of some organs in the larva also must remain doubtful until the further development is known. Our failure to rear the larvæ of Aplysia beyond the free-swimming stage renders the present work very incomplete, and it is therefore the intention of one of us to attempt to continue it and follow the metamorphosis. There would seem to be some hope of success if the methods of prepared sea-water and special feeding were used, such as have been employed so satisfactorily at Plymouth in rearing Echinoderms.

The living embryos are very opaque, and little can be seen of their organisation. As was the case with the work done previously on the cell-lineage, our observations were all made from preserved material. The eggs are enclosed in gelatinous capsules, and these are suspended in a long thread of jelly. Carazzi made the following calculations:—there are on an average seven eggs in each capsule in A. punctata and fifty in A. limacina; the whole thread, or "nest," as he calls it, will therefore contain on an average 80,000 eggs in the former species and 2,000,000 in the latter; this last number may at times be as high as 3,000,000. All the eggs develop with the exception of a few, which are not fertilised or are abnormal from some other cause. In the later stages, when movement is active and the muscles fully developed, the embryos will contract very considerably on the addition of the fixing agent, and this renders them difficult to interpret. To avoid this a 2 per cent. solution of cocaine in sea-water was used, which narcotises them in a few minutes and makes it easy to obtain preparations of fully-extended embryos. It is troublesome, and takes much time to extract the embryos alive from their capsules, and the great majority get injured in the process. Most fixing agents do not harden the jelly, and it is therefore equally difficult to extract the embryos when fixed by most of the common means. Formol, however, has the effect of hardening the jelly, and it is on this account extremely useful. Alone it makes a good fixing agent, but subsequent staining is rendered easier if it is used in combination with some other fixative. At the suggestion of Prof. Meyer a solution of formol and picric was used, made up in the following way:—ten parts 40 per cent. formol, ten parts 1 per cent. picric, eighty parts sea-water. This proved to be by far the best of all the fixing agents which were tried, though for special purposes others were used, as, for example, Hermann's fluid to show up the liver.

When a thread of spawn was laid it was taken and suspended in the tank or jar by means of a string. When the embryos had reached a stage which it was desirable to preserve, a piece an inch or so in length was cut off the end of the thread, divided into a number of fragments a few millimetres long, and put in the picric and formol solution for about half an hour. At the end of that time it was easy to break the capsules with a needle and extract the embryos, the greater number of them entirely uninjured. Various stains were used, but paracarmine gave the best results for whole preparations; sections were stained on the slide with borax-carmine, followed by picro-indigo-carmine.

The eggs of Aplysia are small, being less than 100μ in diameter, and this makes orientation before section-cutting practically impossible. In the end, therefore, it was found more convenient to embed large numbers close together which could all be cut at the same time, for in this way one could be certain of getting a few embryos cut in the plane that was desired. In order to embed a large number of eggs in a small area of paraffin the following method was employed—a watchglass was filled with paraffin and allowed to cool; a small round hole, reaching at least half way through the paraffin, was then made; the embryos were transferred into this by means of a fine pipette, and as much xylol as possible drawn off. The watch glass was then placed for half an hour on a stand on the warm bath, for half an hour on the bath itself, and finally inside the bath until the paraffin melted completely, when it was cooled.

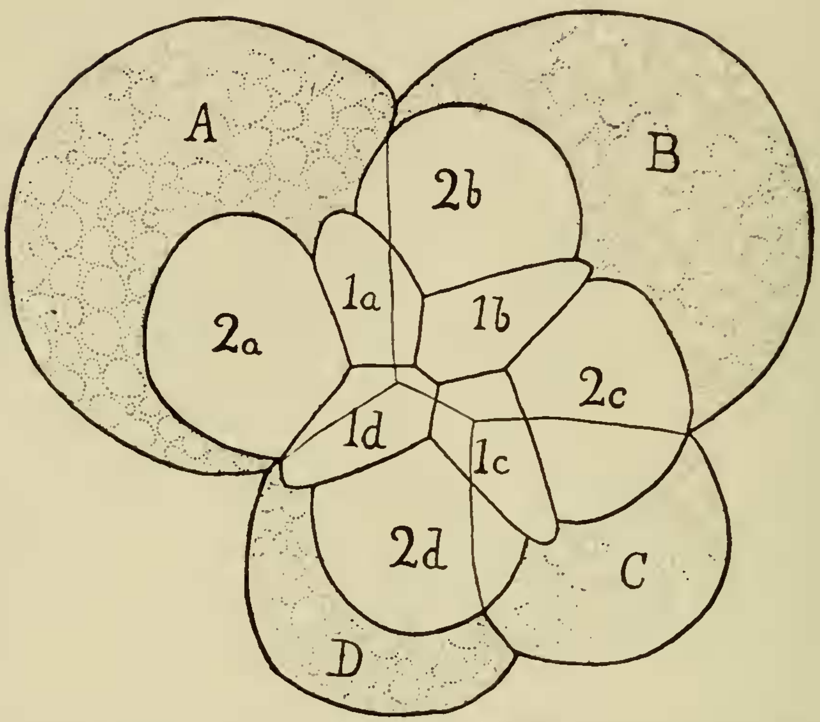
More points remain undecided in the ontogeny of Molluscs than perhaps in any other group in the Animal Kingdom. The cell lineage has been worked out in numerous cases among the various groups, but our knowledge of those stages which follow upon the end of segmentation is very incomplete.

This work was undertaken to throw light, if possible, on the origin of kidney, heart and pericardium, about which the most diverse statements have been made. It will suffice here to point out that at least six different types of excretory organs have been described in Molluscs, and that the origin and homology of all of them is disputed; while as regards the cœlom, opinions differ equally widely. The bearing of our results on these questions will be discussed at the end of this paper. Owing, however, to the fact already mentioned, namely, that it has not yet been found possible to rear the larvæ through the metamorphosis, they remain for the present inconclusive.

For the purpose in view, Aplysia was chosen for two reasons, firstly because it is easy to obtain material at any period of the year, and secondly, because a very careful and complete account of the cell lineage has been given by Carazzi. It was hoped that by beginning at the point where Carazzi left off, it would be possible to follow the development of the organs, and definitely to ascertain from which cells they arose. To the excellent account of the cell lineage referred to we have nothing to add; every cell has been followed up in it to a time when there are more than one hundred, and the history of the endoderm and mesoderm has been traced further. His last description is of an embryo consisting of two hundred and fifty cells, with the velum already developed.

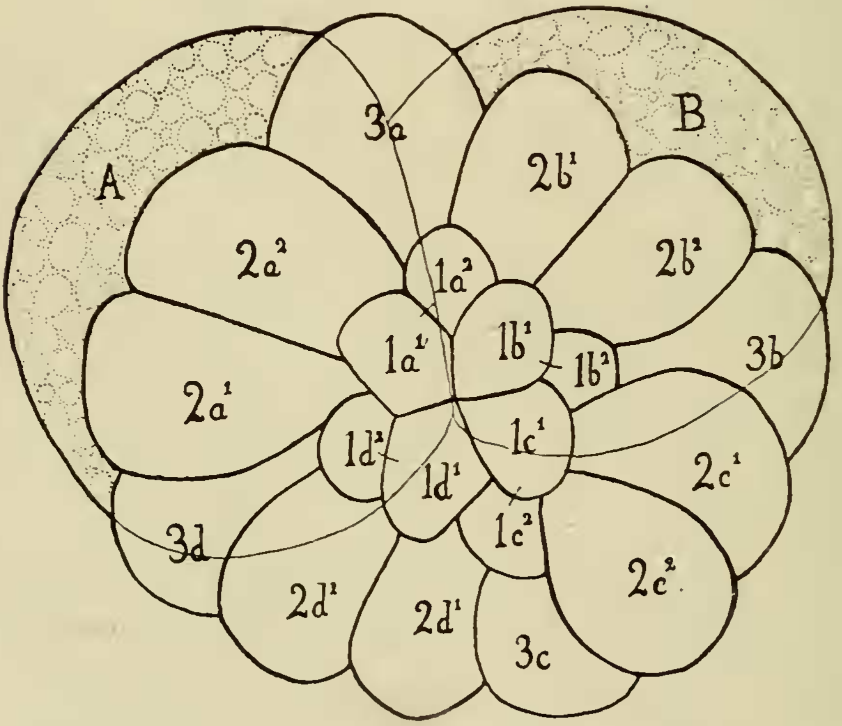
The development as described by Carazzi may be summarised as follows: Segmentation is spiral, dexiotropic and unequal, the endomeres *A* and *B* being far larger than *C* and *D*. The great size of these cells makes the cleavage look at first very irregular, but as a matter of fact their destinies show no exceptions to the scheme which has come to be recognised as normal in eggs the segmentation of which is of the spiral type. The first three quartettes give rise to all the ectoderm, 4 *d* entirely to mesoderm. There is no larval mesoderm arising from the ectoderm as has been described in some forms. The endoderm is derived from 3*A*, 3*B*, 3*C* and

TEXT-FIG. 1.



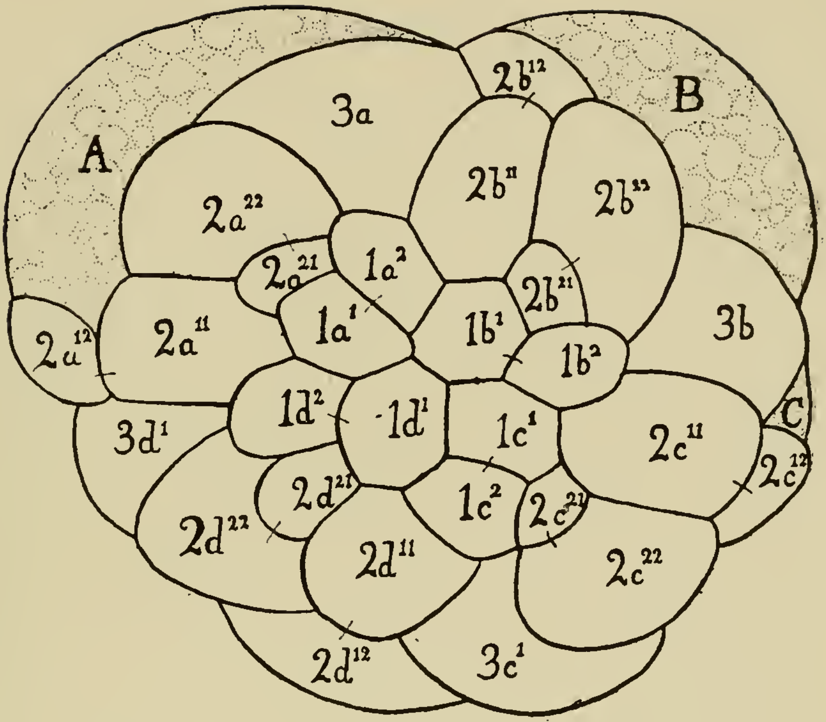
Surface view from the animal pole of an egg in the 12-cell stage.

TEXT-FIG. 2.



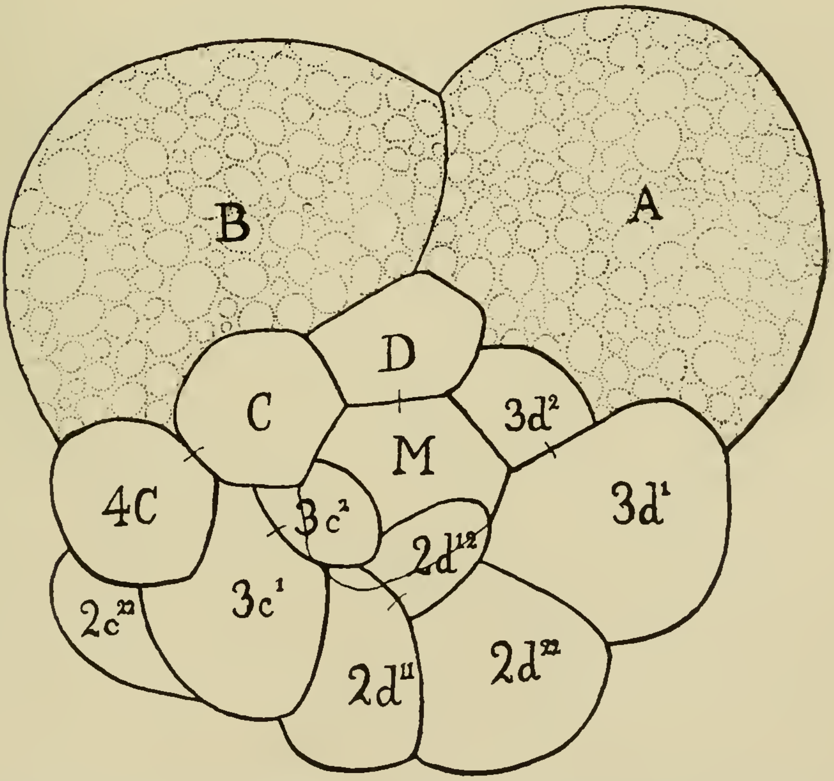
Surface view from the animal pole of an egg in the 24-cell stage.

TEXT-FIG. 3.



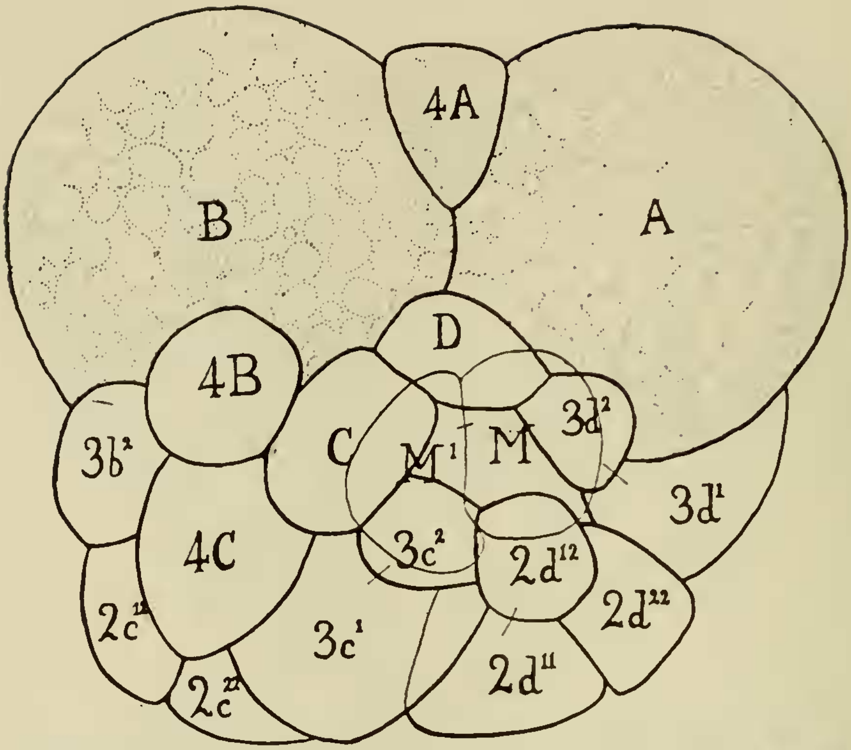
Surface view from the animal pole of a later stage; the apical ectoderm is now formed from 8 cells of the first quartette of micromeres, 16 of the second, and 6 of the third; $2a^{21}$, $2b^{21}$, $2c^{21}$, and $2d^{21}$ are the tip cells of the apical cross.

TEXT-FIG. 4.



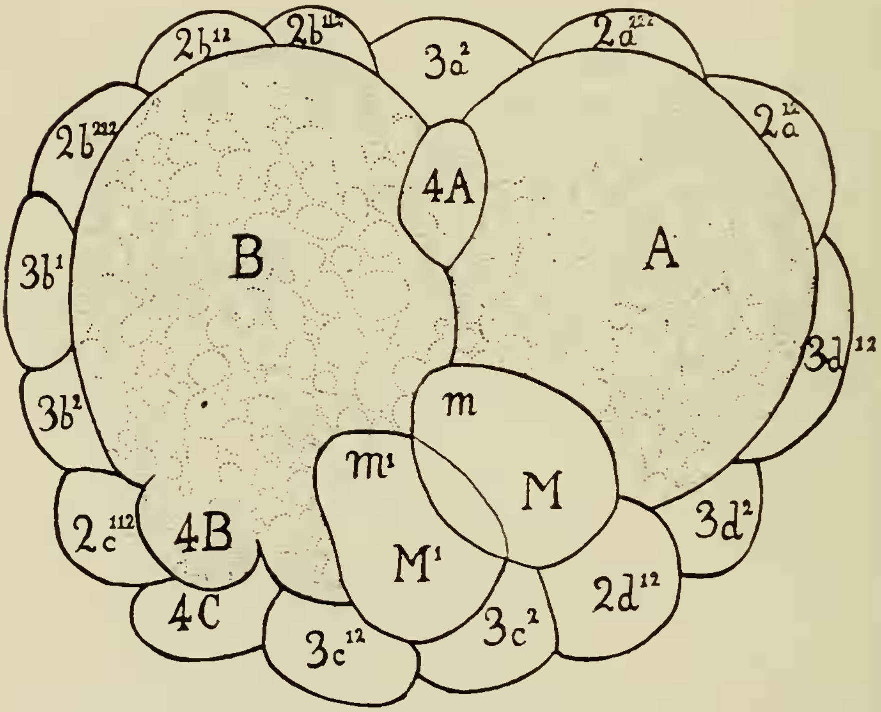
Egg seen from the vegetative pole at a stage corresponding to Text-fig. 2. The mesoteloblast *M* is already formed by the division of *D*.

TEXT-FIG. 5.



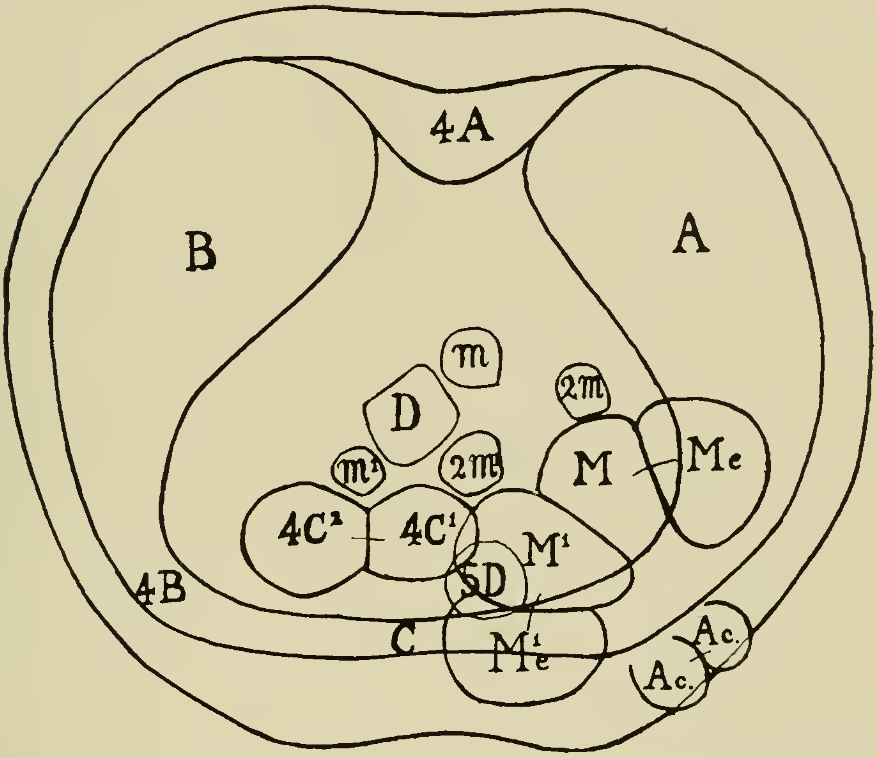
Egg seen from the vegetative pole at a later stage. M has divided to give rise to the paired mesoteloblasts M and M^1.

TEXT-FIG. 6.



Optical section of an egg at a somewhat later stage at the level of the mesoblasts, seen from the vegetative pole. The two cells, M and M^1, have each given rise to a small anterior mesoderm cell, m and m^1.

TEXT-FIG. 7.[1]



Diagrammatic optical sagittal section, seen from the vegetative pole of an egg in the 170-cell stage. The derivatives m, m^1, $2m$, $2m^1$, of the mesoteloblasts M, M^1, are seen spreading in an anterior direction from the region of the anal cells $Ac.$ The macromeres A and B have diverged from one another to form a segmentation cavity.

[1] This and the preceding six text-figures have been modified from Carazzi's drawings. The notation of the various blastomeres throughout the segmentation follows the system, now almost universally adopted for the description of cell-lineages, of Wilson ("The Cell-lineage of Nereis," 'Journ. Morph.,' vi), slightly modified by Conklin ("The Embryology of Crepidula," 'Journ. Morph.,' xiii). A, B, C, D are the macromeres from which the successive quartettes of micromeres are divided off; the quartettes being distinguished by the co-efficients 1, 2, 3. Thus the first quartette will consist of $1a$–$1d$, and its derivatives will be $1a^1$, $1a^2$, $1d^1$, $1d^2$; while the descendants of the latter generation will be $1a^{1\cdot1}$, $1a^{1\cdot2}$, $1a^{2\cdot1}$, $1a^{2\cdot2}$–$1d^{1\cdot1}$, $1d^{1\cdot2}$, $1d^{2\cdot1}$, $1d^{2\cdot2}$. By the division of D $4d$ is formed, which, since it contains the material for the production of the mesoderm, is designated by the letter M. This later divides to form the mesoteloblast M and M^1, and from these, after the separation of a mesentoblast from each, small cells m and m^1, etc., are budded off

4D; gastrulation is of the epibolic type, and the blastopore is formed at the vegetative pole. It narrows to a slit-like opening, diminishing in size by the continual growth of the anterior and lateral parts of the ectodermal sheath, but does not close completely, but persists as the mouth. At the end of segmentation, owing to the large size of the endomeres *A* and *B* the embryo becomes somewhat heart-shaped. Between the large endomeres a small space appears, the segmentation cavity, which is more or less triangular in shape in optical section, the broadest end being towards the posterior end of the embryo. Two ectoderm cells, $2d^{22221}$ and $2d^{22222}$, increase greatly in size and come to project from the surface. These are known as the anal cells. During segmentation there is a shifting of the embryonic axis, and these cells come, in consequence, to mark the posterior end of the larvæ. At the opposite end the velum is formed as a simple ring in the region of the *B* quartette. By the time the cilia are developed the embryos begin to rotate within their capsules. At this stage there are about two hundred and fifty cells.

Text-fig. 7 represents an embryo with about one hundred and seventy cells, seen in optical section, from the vegetative pole. The blastopore is now reduced to a narrow slit, and posteriorly the anal cells project from the surface. Anteriorly the polar bodies were present still adhering to the embryo, but are not represented. Internally the two large endomeres diverge from one another to leave the segmentation cavity between them, while the derivatives of the much smaller endomeres *C* and *D* are shown. The mesomeres, which are at this stage eight in number, stretch across from the anal cells towards the position occupied by the blastopore, which is not represented.

anteriorly to give rise to the mesodermal bands. After the formation of the three quartettes of micromeres a fourth generation is produced by *A*, *B*, *C*; this consists of *4A*, *4B*, *4C*, which go towards the formation of the endoderm. The above lineage is given in tabular form by Robert (**26**), to which the reader is referred for the detailed analysis of the later segmentation stages.

We propose first to describe in some detail the earliest stage which we have investigated, and then to follow the development of the various organs separately up to the time when the larva becomes free swimming.

Structure of the Embryo at the End of Segmentation.

Pl. 22, fig. 1 is an external view of an embryo shortly before rotation begins, and fig. 2 in the same plate shows the cells which have sunk below the surface at the same stage. The total number of cells is more than 300. The ectoderm forms a thin and uniform layer covering the surface of the embryo. Round the anterior end there is a ring of somewhat larger cells derived from the *B* quartette, which bear long cilia and form the velum. At the opposite pole are the two anal cells $2d^{22221}$ and $2d^{22222}$, which are very prominent and project markedly from the surface, thus forming a convenient means of orientating the embryo with certainty. Near their bases are small nuclei which are sometimes difficult to see, and were not noticed by Blochmann (**2**). Their cytoplasm is very much vacuolated. They presumably function as temporary excretory organs. They are characteristic of Opisthobranch larvæ, though in some cases, as in Fiona, described by Casteel (**6**), they are small and but little differentiated from the other ectoderm cells. It is well known that in certain other Gastropod larvæ ectoderm cells of considerable size are found projecting from the surface; Glaser (**8**) has described them in Fasciolaria, where they occur singly or in groups of two or three together. These would appear to be comparable to the anal cells of Aplysia, but in Fasciolaria their position is variable, being almost anywhere on the surface. In the region of the A quartette there is a slight projection, the cells being somewhat enlarged. This is the rudiment of the foot. Between the foot and velum is the blastopore on the ventral surface, and round it the ectoderm cells are beginning to sink in. Though the blastopore is at this time very small, we have always found it perfectly distinct, and in this we agree with Carazzi in contradiction to Mazzarelli and

Blochmann, who assert that it closes and then reopens to form the mouth. The stomodæum does not at this stage communicate with the space between the endomeres; it is a blind sac lined by ectodermal stomatoblasts and œsophagoblasts. The former, according to Carazzi, are derived from $3a^1$ and $3b^1$, and the latter from $3a^2$ and $3b^2$; together they number between twenty-five and thirty. On the dorsal surface of the embryo and posterior is the shell-gland. It consists of a deep and narrow invagination, formed by a large number of cells, which are slightly differentiated from the adjacent ectoderm by their more elongated shape and rounder nuclei.

On each side of the embryo, just ventral to a line joining the anus and the mouth, a small ectodermal invagination, *ot.*, is seen to be in process of formation. These are the pair of otocysts. Fig. 3 shows the same structures at a slightly later stage. A little anterior to the anal cells on the right side are four large ectoderm cells identified by Carazzi as $3c^{1111}$, $3c^{1112}$, $3c^{1211}$ and $3c^{1212}$. These cells are at this time clearly in the ectodermal layer, but they soon sink below the surface and give rise to the secondary kidney. Their nuclei are of great size, and generally each contains one prominent darkly staining plasmosome.

The greater part of the interior of the embryo is occupied by the two large endomeres *A* and *B* (not lettered in the plate). They diverge somewhat from one another, and thus enclose between them an irregularly triangular segmentation-cavity (marked *st.* in figs. 2 and 3). The broad end abuts upon the shell-gland posteriorly, while the narrow end reaches to the bottom of the still blindly-ending stomodæum. The nuclei of the endomeres are large and oval in shape, lying to the inner side of the cells near the segmentation cavity. The cytoplasm is heavily laden with large yolk-granules, and some of the yolk is often found in the nuclei also, causing the latter to stain very deeply with plasma stains. A large vacuole is generally present in each of the endomeres, which is very conspicuous in the living embryo, and persists for a long time.

At this period there are about twelve other endoderm cells, the derivatives of *4a* lying close against the stomodæum in the anterior end of the segmentation cavity, those of *4b* at the opposite end rather dorsal to the shell-gland, and *C* and *D*, with their descendants, also lying at the posterior end against the wall of the shell-gland. The latter are already beginning to form a fairly definite row, which will become the posterior well of the stomach. At this stage the greater part of the cavity, which will be the stomach, is bounded only by the endomeres *A* and *B*, but this soon ceases to be the case. *A* and *B* gradually take less and less part in the formation of the wall and give rise to the left liver.

There are between fifteen and twenty mesoderm cells in the embryo at this stage. They form an irregular band, which arises at the posterior end near the anal cells, and stretches forwards to the blastopore. The band lies chiefly on the right side, but certain cells are already beginning to pass dorsally and ventrally into the foot.

We here see that it is possible to speak of a mesoderm band in Aplysia, though it is never clearly defined and soon breaks up. The conditions are very much like those described in Fiona and Umbrella, though the great size of the endomeres in Aplysia has forced the mesoderm chiefly on to the right side.

There is no secondary mesoderm either in this or later stages. It is present, however, in Fiona. Unfortunately, Heymons worked on Umbrella at a time when the existence of secondary, or ecto-mesoderm, was not recognised, so that its presence or absence in that form is unknown.

DEVELOPMENT OF THE ORGANS.

The embryo rapidly assumes the appearance of the free-swimming larva, and from the beginning of rotation onwards there is but slight alteration in shape and very little increase in size until just before the embryo emerges from the capsule.

The Velum.—The velum is, as we have seen, originally a simple ring of cilia round the anterior end of the body (Pl. 22, fig. 4). As the anterior end, however, grows out within the velar area, and then becomes flattened and expanded laterally, the circular shape of the ciliated band is soon lost, and the latter comes to surround the widely extended anterior prolongation of the body (Pl. 22, fig. 6). The velum then becomes notched in the mid-dorsal line and bilobed, but the latter characteristic is not so well marked as is generally the case in Opisthobranch larvæ.

In the free-swimming veliger the full extension of the velum is reached, but it can always be contracted completely within the shell. The cilia are long and prominent. Inside the circle of these cilia-bearing cells is a second row of cells of rather larger size with three or four cilia each, and in the middle, a cell with a single long and prominent flagellum.

The Foot.—The rudiment of the foot is at first broad and blunt, projecting from the ventral surface of the embryo between the blastopore and the anal cells. There is no sign of a division into two, as has been described in early stages of Patella. Between the stages represented in Pl. 22, figs. 4 and 5, the foot has undergone considerable change in shape, becoming elongated in an antero-posterior direction and flattened dorso-ventrally, and the operculum has been secreted on the lower surface. In the free-swimming larva it is still longer and covered with short cilia, and the operculum is capable of closing the opening of the shell completely when the animal is retracted.

The Shell-gland.—In Pl. 22, figs. 2 and 3, the shell-gland is invaginated to form a narrow pit. It soon afterwards becomes everted, and fig. 4 of the plate shows the posterior end of the embryo covered with a thin shell. The cells that were invaginated now form a cap, which secretes the shell, the edge of the former becoming the edge of the mantle. The mantle-cavity in the free-swimming larva is fairly deep, and into it on the right side open both the anus and the secondary kidney.

The Shell.—This is secreted directly the shell-gland is everted. It is at first very thin and transparent, and even at its fullest development in the free-swimming larva never becomes thick or resistant enough to interfere with section-cutting. It grows at once into its ultimate exogastric form, and is always perfectly symmetrical. In the free-swimming larva it is marked by a number of fine lines, forming an irregular network.

The Anal Cells.—These have been already described (*Ac.* in Pl. 22). As development proceeds they decrease in size, this reduction being probably correlated with the growth of the secondary kidney, which takes on the function of excretion. In the free-swimming larva they are still prominent features, though they are neither figured nor described in this stage by Mazzarelli. They presumably disappear towards the end of the larval period.

The Otocysts.—These arise as ectodermal invaginations of about six cells, one on each side of the rudiment of the foot (*ot.* in Pl. 22). Later some ten or twelve cells sink well below the surface and form closed vesicles of some size, which are very obvious in the living larva lying at the base of the foot, below and to the sides of the œsophagus. At first these vesicles seem to be empty, but towards the end of embryonic life a large spherical otolith is very conspicuous inside each.

The Nervous System.—We have seen no trace of the nervous system before a stage corresponding to Pl. 22, fig. 8. In such an embryo there are visible rudiments of both cerebral and pedal ganglia (*c.g.* and *p.g.*). Our preparations do not make the mode of origin of the nervous system very clear. It would appear to arise as a cell-proliferation from the ectoderm, as there is no evidence of an ectodermal invagination to form the ganglia, as occurs in some forms, Dentalium for example. When the ganglia first appear, they take the form of slight thickenings in close contact with the ectoderm. The cerebral ganglia lie just above the mouth, the pedal ganglia to the outer and ventral sides of the otocysts, and slightly anterior to them. The ganglia become more definite and larger, but in the free-swimming larva they are still near the surface. The two cerebral ganglia are close together and are united by a broad commissure. Mazzarelli states that cerebro-pedal and pedal commissures are present. We have been unable to discover these. The velum and foot are at this stage full of connective tissue, and it would be difficult to trace a fine commissure if it did exist. Visceral ganglia are absent at this stage of development.

The Secondary Kidney.—In making use of the term "secondary kidney" we are following the nomenclature of Mazzarelli. In his study of the free-swimming larvæ of Opisthobranchs he gives this name to the unpaired right kidney, which he has shown to be characteristic of all these larvæ. The term "primitive kidney" he reserves (and we follow him in doing so) for the smaller paired kidneys, the nephrocysts of Trinchese, which lie anteriorly to the "secondary kidney" at the base of the velum. It is necessary to make this clear, since owing to the nomenclature used in Carazzi's recent work confusion may arise. In the earliest stage with which we deal we have already described four large ectoderm cells, and have said that they would give rise to the secondary kidney. Now Carazzi mentions these cells and identifies them as $3c^{1111}$, $3c^{1112}$, $3c^{1211}$, and $3c^{1212}$. We have no doubt that these four cells are the same as those which we describe and figure. But Carazzi in his table of the cell lineage marks these cells as giving rise to the "reni primitivi." In the text all he says with reference to the fate of these cells is: "Una parolo devo aggiungere sul desterio delle grandi cellule $3c^{1111}$ $3c^{1112}$; esse constituiranno uno dei primi organi emissionali, civè il rene primitivo." It is quite impossible from this to understand whether, as one would incline to think from the quotation cited, Carazzi calls primitive kidney what we call secondary kidney, or whether, from the fact that in the table of cell lineage he says these cells give rise to "reni primitivi" (in the plural), he has not made the mistake of thinking that the cells in question are the rudiment of what we call primitive kidneys, and not of what we call secondary kidneys.

These four cells become differentiated while in the ectodermal layer on the right side of the embryo and slightly in front of the anal cells. Even in the earliest stages, which we have examined, their nuclei are clearly to be distinguished from all the other nuclei in the embryo, not only by their much larger size, but by the presence in almost every case, of a conspicuous deeply-staining plasmosome. Text-fig. 15 is a section of a stage where these cells have just begun to sink below the surface. Of the two cells shown one is still in the outer ectodermal layer, while the other has already sunk below. This process has gone further in Text-fig. 16 and the four cells are covered by a thin ectodermal layer. They continue to sink in further, and gradually give rise to a compact pear-shaped organ, the apex of which is directed towards the surface. Text-fig. 17 is a section taken at a stage when the organ is first becoming definite. We have never seen any of the four cells in the process of division; but the kidney in the free-swimming larva consists of eight cells, and therefore each original cell must divide once. The cytoplasm is at first finely vacuolated, but as development proceeds the small vacuoles become confluent, and form in the external part of the kidney several large cavities, the narrow ends of which converge to a point where they open into the mantle cavity. Here two small ectodermal cells form a short duct (Text-fig. 19). In the living larva drops of coloured liquid are seen to be contained within the vacuoles, but as a rule they are dissolved out by the reagents used in the course of preservation. The whole organ is clothed with a thin mesodermal epithelium.

This single excretory organ has long been known in Opisthobranch larvæ, but the most diverse statements have been made both as to its origin and function. With regard to the latter point, there can be no doubt that it is an excretory organ since it is easy in the living larvæ of some Opisthobranchs to observe the process of excretion. The view put forward by Lacaze-Duthiers and Pruvot (**13**) that it was an "anal eye" must be held to be one of the most curious and unwarranted of zoological speculations. Two investigators, Casteel working with Fiona and Heymons with Umbrella, have come to the same conclusions as we have regarding the origin of this organ. In Umbrella it is originally paired. There are large ectodermal cells, $3c^{11}$ and $3d^{11}$, on either side of the embryo which divide and sink below the surface, one cell in each group remaining especially large. The cells on the left later disappear, while the right group forms the kidney. In Fiona the secondary kidney is unpaired from the beginning, as it is in Aplysia. It consists, however, of a single cell, $3c^{111}$. This closely resembles the cells which form the organ which we have described in Aplysia, the cytoplasm being much vacuolated, and the nucleus large and containing nucleoli. In this case there are also other ectoderm cells near by, which seem to function in the same way. Clearly, we are dealing with a very similar organ in these three forms, but in Aplysia it is better developed, forming a definite organ with a duct and an enveloping epithelium.

The ectodermal origin of this kidney was first recognised by Lacaze-Duthiers and Pruvot. Mazzarelli is the only recent writer who upholds the view that it is mesodermal. He has worked on Aplysia and a number of other Opisthobranchs, and has come to the same conclusion for them all. We find his observations difficult to reconcile with our own. The organ in question, according to his account, is derived from two mesodermal cells at the aboral pole, which represent a paired rudiment of the kidney, as in Umbrella; in the course of torsion, however, both cells get pushed round on to the right side and form the single unpaired structure. They divide, become surrounded by other smaller mesodermal cells, and finally come to communicate with the exterior by an ectodermal invagination. It would seem that he took for the rudiment of the kidney two of the large mesoderm cells, which lie, at the stage he describes, on either side of the aboral pole; the large ectodermal cells, still lying at the surface, he has apparently overlooked. But why at a later stage he should describe two cells, when there are never less than four present, it is not easy to explain.

The Primitive Kidneys.—These organs, described by Trinchese as nephrocysts, consist each of a large, much-vacuolated cell with a small nucleus, lying one on either side of the body at the base of the velum. In the living embryos they are very obvious on account of the brightly-coloured oily globules which they contain (Pl. 22, *K* 1, in figs. 4 to 9). They appear to be characteristic of Opisthobranch larvæ, and presumably constitute temporary excretory organs. Mazzarelli ascribes to these cells a mesodermal origin, and when first seen they certainly appear to lie well inside the body in both Fiona, according to Casteel, and Aplysia. All previous attempts to trace them back to their origin in segmentation have failed, and in spite of employing various methods of preserving and staining we have been equally unsuccessful. We consider it probable, however, that they are of ectodermal origin, of the same nature as the anal cells, which sink below the surface and lose their excretory function at a time when the secondary kidney is developed to take it on.

The Alimentary Canal.—We left the segmentation cavity at a stage when it was largely bounded by the two large endomeres, *A* and *B*. Pl. 22, fig. 3 represents a slightly later stage. The number of endomeres has increased, not by the division of the large blastomeres, *A* and *B*, but of the smaller endoderm cells. In the anterior region of the segmentation cavity the two large endomeres do not, therefore, contribute to form its boundary to such an extent as before. The cells which give rise to the wall of the cavity in this region are chiefly derived from 4*a* and *D*. The posterior wall of the cavity is still more complete, and its constituent cells are the derivations of 4*b*, *C*, and *D*. This corresponds very closely with the condition of things in Fiona, where the posterior wall is formed by 5*B*, 5*b*, 4*c*, 5*C*, 5*c*, 4*D*, and 5*A*. Umbrella agrees very nearly in this respect with Aplysia and Fiona.

At a slightly later stage (Pl. 22, fig. 4) the stomodæum breaks through and comes into communication with the segmentation cavity. When this occurs, the anterior wall of the latter is still in part formed by the two endomeres, *A* and *B*. This, however, soon ceases to be the case. At this stage the intestine grows out as a tube-like evagination from the right posterior portion of the stomach, and reaches the surface just behind the anal cells. The anus is formed at once, and there is but a very slight ectodermal invagination, forming only the lip of the aperture. In this respect Aplysia agrees exactly with Umbrella. In this form the intestine arises from the derivatives of 5*c* and 5*d*; and this is probably the case also in Aplysia.

The œsophagus is long and narrow, and almost entirely ectodermal. From an early period it is ciliated, in the free-swimming larva the cilia being very long and numerous, and in sections filling up almost the whole lumen of the tube. Mazzarelli describes a cuticle which lines the cavity of the œsophagus of Opisthobranch larvæ; our preparations of Aplysia certainly do not show this structure.

The stomach wall is formed by rather small, clearly defined ciliated cells, constituting a columnar epithelium. In the embryonic stages the cilia are all alike throughout the lining of the cavity, but in the free-swimming larva, in the posterior region they are replaced by stiff hair-like structures, which Mazzarelli calls "bastoncelli." They are probably fused cilia, and serve as a staining apparatus. The fact that they are not developed until the larva becomes free-swimming supports this view, for until that stage is reached the embryo feeds upon the yolk stored in the liver, and does not take in food through the mouth. No epithelium can be seen covering the wall of the stomach externally. The intestine is a simple ciliated tube, and also appears to lack an epithelial investment. At first (Pl. 22, fig. 4) it arose from the right ventral posterior region of the stomach, but in the course of the torsion, which affects the whole of this part of the body, it becomes carried up on to the right side (Pl. 22, figs. 6 and 7); and finally, in the free-swimming larva it is seen to pass from the dorsal surface of the stomach, slightly to the left of the middle line. The anus opens into the mantle cavity a short distance below the secondary kidney.

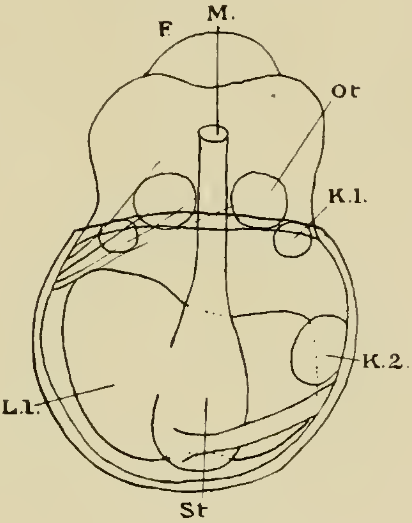
It will here be convenient to say a few words about the torsion which the embryo undergoes in the course of its development. This involves two processes, perfectly distinct from one another, though in Aplysia they take place simultaneously, one being the oro-anal flexion, so characteristic of molluscan organisation, and the other the rotation of the anus and adjacent organs through more than 120°, round an axis coinciding with the antero-posterior axis of the embryo. By the former process the anus is carried forward to open anteriorly, and the intestine to lie ventral and parallel to the œsophagus; while by the latter, torsion, properly so-called, the intestine, liver, kidney, and cœlom are carried from the ventral surface up on to the right side of the body. This movement is marked externally by the change in position of the anal cells (Pl. 22, figs. 4, 5, 6, 7, 8). The shell alone appears not to be affected by the torsion, for before this process is complete it has in miniature assumed its final shape, which it retains, while the organs inside it are being twisted in the manner described.

The Liver.—The endomeres *A* and *B* which form the left liver remain very large, and for a long time do not divide. At a stage corresponding to fig. 5 the nuclei divide, and thenceforward multiply slowly; but for some time no corresponding cytoplasmic divisions are to be distinguished. The nuclei are at first large and filled with yolk, scattered amongst irregular fragments of chromatin. Later they become reduced in size after repeated divisions and lose their yolk contents. The endomeres are full of yolk-granules, and their cytoplasmic structure is thereby entirely obscured. Of the two, *B* is approximately dorsal and *A* ventral. The effect of torsion is to move *B* more over to the left and *A* slightly to the right. They remain perfectly distinct from one another for some time, but eventually become fused together on the left side to form a single organ, the left liver. We have spoken of the two large irregular spaces generally to be seen in the two endomeres. These coalesce and form a cavity within the liver, which communicates with the stomach by an irregular gap in the wall of the latter on the right side and rather ventrally. Text-fig. 20 is a section taken through this gap. On the left side is seen the cavity of the stomach communicating with the cavity of the liver; on the right side is the right liver, to be described later on. The nuclei have divided and are reduced in size. The size and shape of the liver in the free-swimming larva can be made out from Pl. 22, figs. 8 and 9. It may roughly be considered to consist of two lobes, of which the left is derived from *B* and the right from *A*. The left lobe is much the largest; it covers the left wall of the stomach, projects anteriorly to it, and rises dorsally. The right lobe lies ventrally, projecting beyond the stomach on the right side. The liver is clothed externally by a fine epithelium of flattened cells. During the whole of the embryonic period the yolk is being consumed, and when the larva emerges from its capsule it is entirely used up. The liver now takes on a new function, presumably one of secretion and digestion, for we have often observed algæ and other food material in the cavity, and drops of secretion are at times to be seen in the cells.

The right liver is formed in an entirely different manner. At a time between the stages represented in Pl. 22, figs. 4 and 5, certain cells of the right anterior wall of the stomach, rather nearer the dorsal than the ventral surface, become pushed out to form a flat, knob-like process (*R. l.*). This gradually takes on a rounder shape, and the contained cavity increases in size, while it becomes constricted off from the stomach to form a definite organ, which is the right liver. In the free-swimming larva it is nearly round, and communicates with the stomach only by a small and somewhat irregular aperture. When fully formed it lies almost entirely dorsal to the stomach, the smaller retractor muscle passes above it and the intestine below and to the right. The cells composing it are of large size, the cytoplasm consisting of a network enclosing uniform small vacuoles which never contain yolk. The nuclei are not very different in appearance from those of the stomach wall, but are usually larger in size. Externally it is clothed with a thin epithelium. The right liver undergoes no apparent change when the larva emerges from its capsule. It is presumably always secretory in function.

Our account of the development of the liver differs from that of Mazzarelli, for he states that the right and left liver arise each from one of the two large endomeres, and this we have shown to be incorrect. The peculiar mode of formation of the left liver is evidently correlated with the great size of the endomeres. All the yolk is contained within them, whereas, in such a form as Fiona, certain cells of the anterior portion of the stomach wall derived from $4b^2$ are heavily laden with yolk and become gradually evaginated to form the liver. This mode of formation of the liver in Fiona is, in fact, not unlike that of the right liver in Aplysia, but the latter, as we have pointed out, never contains yolk.

TEXT-FIG. 8.



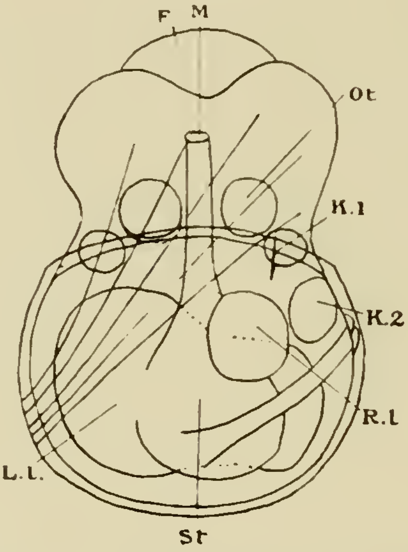
Diagrammatic view of an embryo at a stage when the large retractor muscle is first formed; seen from the dorsal surface. *F.* Foot. *K* 1. Primitive kidney. *K* 2. Secondary kidney. *L.l.* Left liver. *M.* mouth. *Ot.* Otocyst. *St.* Stomach.

The large liver is very characteristic of Opisthobranch and

Pteropod larvæ, and serves to distinguish them from other Molluscan embryos. There may be present only a single liver, as in Fiona, or there may be also a smaller right lobe, as in Aplysia.

Muscles.—These are two in number, one large and conspicuous and the other much smaller. The former makes its appearance at a stage slightly earlier than that represented in Pl. 22, fig. 6, when it consists of two or three fibres only, which arise from scattered mesoderm cells descended from 4*d*.

Text-fig. 9.

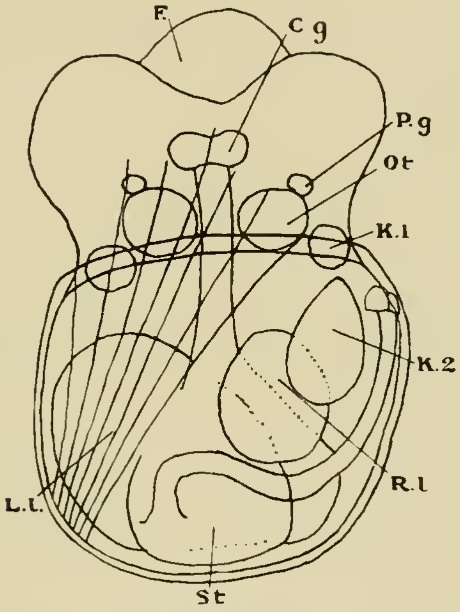


Similar view of an embryo at a slightly later stage. *R.l.* Right liver; other lettering as in Text-fig. 8.

There is no larval mesoderm in Aplysia derived from the quartettes of ectomeres, which in many cases give rise to the larval musculature. The fibres are at first attached to the body-wall dorsally and to the left of the middle line at about the level of the mantle-cavity (Text-fig. 8). At a later stage they are found to have increased in number and to be attached further back and rather more ventrally (Text-fig. 9), while in the free-swimming larva their attachment to the body-wall is posterior, but slightly to the left of the middle line and still nearer the ventral surface (Text-fig. 10).

The fibres are by that time fairly numerous, each one consisting of a single spindle-shaped cell, showing longitudinal striations. They pass dorsally to the left liver and are distributed to the velum and foot, and some are attached to the œsophagus near the mouth. The smaller retractor muscle appears later in development and always consists of three or four fibres only. They are similar to those of the larger muscle, and are no doubt of the same origin. This muscle is attached posteriorly to the dorsal body-wall on the right side, and thence appears to pass into the velum. As Mazzarelli has noticed, there are no other muscular elements of any kind to be seen in the larva.

Text-fig. 10.



Similar view of a free-swimming larva. *C.g.* Cerebral ganglion. *P.g.* Pedal ganglion. Other lettering as in Text-figs. 8 and 9.

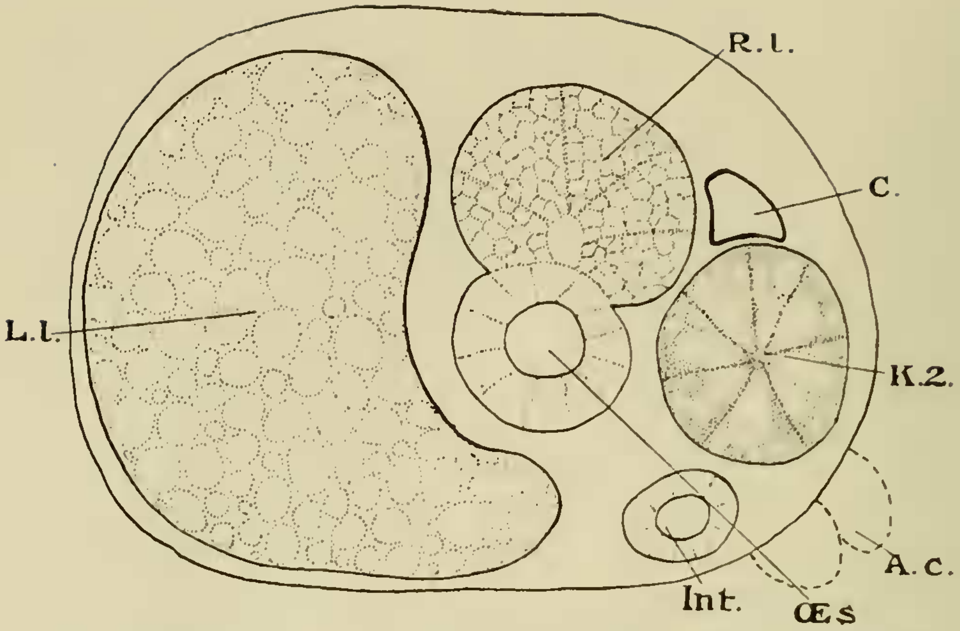
The change in position of the large retractor muscle noticed above is to be attributed to the fact that the left side of the embryo, or more correctly, a particular zone in the left side, grows more quickly than the corresponding zone on the right side. This excess of growth on one side is a familiar feature in discussions of the question of torsion. It was first brought to notice by Bütschli. There is little evidence of its occurrence, but it is most interesting to note that Casteel has stated that in Fiona a portion of the left anterior wall of the stomach can be observed to grow more quickly than the corresponding portion in the right. It is not necessary for us to discuss the theories of gastropod torsion, but we may point out that the excess of growth on one side is merely the ontogenetic cause of torsion. What the original phylogenetic cause of torsion may have been we do not know, and possibly never will know. It is not only possible, but it is probable, that the phylogenetic cause was something totally different from the actual ontogenetic cause. In the more modified members of a group it often happens that certain of the older features in the organisation of the larva get thrown back in development. To some extent this seems to have happened in Aplysia with regard to the torsion, organs seem, that is to say, to develop already twisted. We have indicated the manner in which this occurs with regard to the development of the shell. It must also happen when the visceral loop is developed, for there is no sign of it in the larva when torsion is complete. It must therefore develop already twisted.

The Cœlom.—The mesoderm, as we have seen, appears at first in the form of an ill-defined band, but this arrangement is quickly lost. In Pl. 22, fig. 3 the cells are becoming irregularly scattered, and a little later are to be found everywhere lying between the large yolk-laden endomeres and the ectoderm. In Pl. 22, fig. 2 two small mesodermal cells are seen posteriorly, close to the anal cells; these are e and e^1 of Carazzi, which, he suggests, may possibly give rise to the rudiment of the genital organ. It is probable that these cells become involved in the formation of the cœlom, and that from the wall of the latter at a much later stage the germ-cells arise, but as it is quite impossible to follow these two cells through the larval development, their destiny must remain purely conjectural. As the foot and velum grow out, mesoderm cells pass into them, and there constitute a loose connective tissue; they also form thin epithelial investments to the right and left liver and to the kidneys, and later give rise to the muscles. When the ectoderm cells of the secondary kidney have just sunk below the surface, and before they have become grouped together to form a definite organ, an irregular aggregation of mesoderm cells appears in this region, just anterior to the anal cells (Text-fig. 16). In Text-fig. 17, slightly later, these cells have formed a definite little mass close beside the now clearly developed secondary kidney, and in the next stage (Text-fig. 18) they are seen to bound a narrow slit-like cavity. This is the cœlom. In Text-fig. 19 it has begun to extend anteriorly and dorsally so as to cover the dorsal wall of the secondary kidney and the right and antero-dorsal surface of the right liver. In the last stage, before the emergence of the embryo from the capsule (Pl. 22, fig. 7), the cœlom forms two lateral sacs (coloured red in the figure), that on the right being the larger, connected with one another by two transverse passages—one lying in front of, and the other behind, the right liver, which thus projects dorsally between them. In the free-swimming larva (Pl. 22, figs. 8 and 9) this subdivision of the cœlom has disappeared by the union of the anterior and posterior passages on the dorsal surface of the right liver. The body-cavity now consists of a considerable thin-walled sac, lying on the dorsal side of the larva and covering the stomach, intestine, the right, and a great part of the left liver, and the posterior half of the secondary kidney. Its ventral extension, however, is nowhere very great.

Text-figs. 11–14 show the development of the cœlom in diagrammatic transverse sections through the region of the right liver and the secondary kidney.

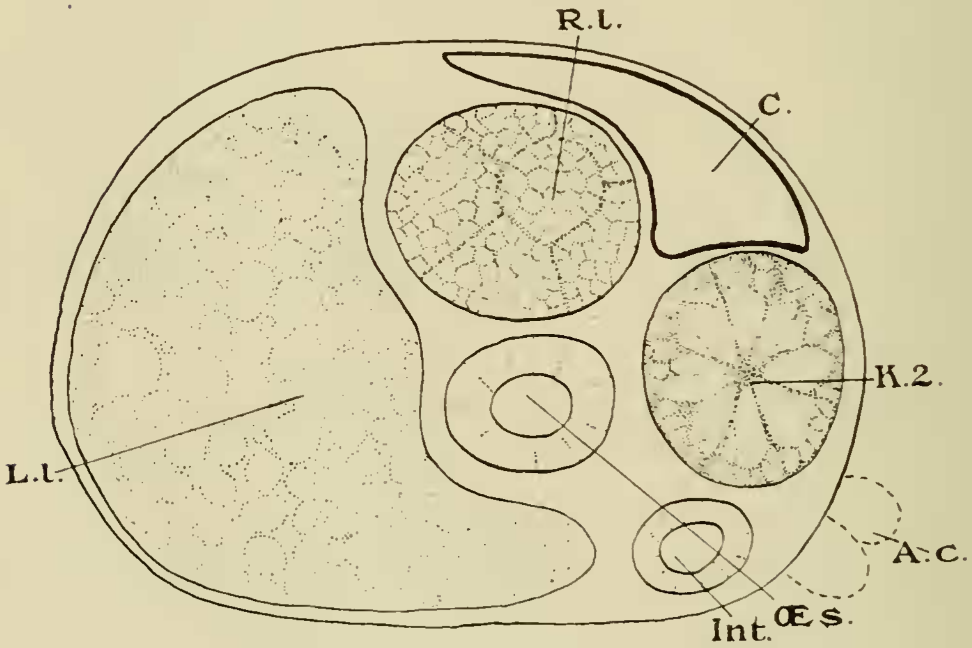
Hitherto the existence of the cœlom in Opisthobranch larvæ has passed unnoticed. Mazzarelli, it is true, mentions a pericardium, which he describes and figures as a small oval sac, but in Aplysia, as we have shown, the cœlom is of considerable extent and irregular shape. It would seem that Mazzarelli only observed the cœlom in whole preparations, which would account for his describing it as a small sac, for

Text-fig. 11.



A.c. Anal cells. *C.* Cœlom. *Int.* Intestine. *K2.* Secondary kidney. *L.l.* Left liver. *Œs.* Œsophagus. *R.l.* right liver.

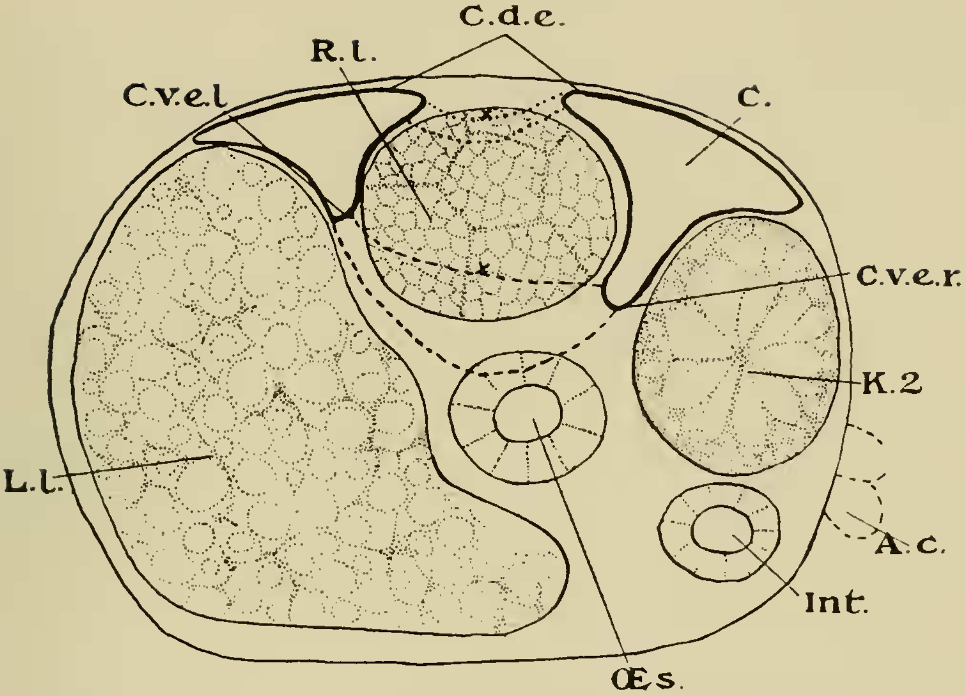
Text-fig. 12.



Lettering as in Text-fig. 11.

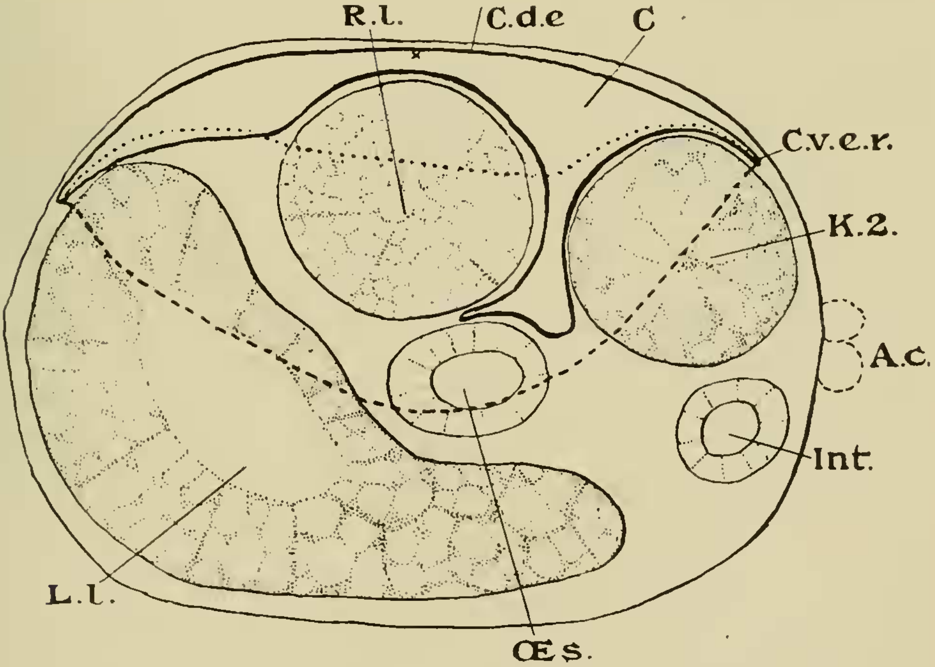
Text-figs. 11–14 are diagrammatic transverse sections through the region of the right liver and the secondary kidney, in order to show the development of the cœlom, which is lettered C.

TEXT-FIG. 13.[1]



C.d.e. Dorsal extension of cœlom. *C.v.e.l.* Ventral extension of cœlom on left side. *C v.e.r.* Ventral extension of cœlom on right side. Remaining letters as in Text-fig. 11.

TEXT-FIG. 14.



Lettering as in Text-figs. 11 and 13.

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[1] The thick broken line indicates the posterior and the thin broken line the anterior extension of the cœlom; the × marks the spot at which the anterior and posterior portions unite dorsally to the right liver.

only the deeper central portion is rendered visible by this method, and does appear somewhat as he figures it, while the processes which spread and pass among the organs can only be reconstructed from sections.

General Considerations.

Although a large amount of work has been done on molluscan embryology, there still remain a number of important questions about which there is no general agreement. One reason for this is that, of the many cases investigated, only a few stages of each are as a rule known. In the majority of cases where the cell lineage has been worked out there is no account of the later stages; and conversely where these stages are well known, the cell lineage has not been traced. Such is the case with Paludina, about the later stages of which form more has been written than about any other molluscan genus. No satisfactory conclusion is likely to be reached until our knowledge of the earlier stages of Paludina is more complete. It was with the object of completing the account of the development in a single genus, in which the cell lineage is known, that we undertook this work. As yet it is not complete for reasons that have been mentioned. But the results that we have so far obtained make it necessary that we should consider their bearing upon certain theoretical points in connection with molluscan ontogeny.

The most important facts in our description of the development of Aplysia are the large extent of the cœlom and the ectodermal origin of the kidneys. And the first of these points bears directly on the question of the relation of Annelids to Molluscs. The resemblance of the trochophore to the veliger larva has been long recognised; there cannot, indeed, be shown to be any essential difference between them. But there are two points in the development of both trochophore and veliger which concern us here—the type of segmentation and the development of the mesoderm. That form of segmentation which is known as spiral cleavage is typical in both Annelids and Molluscs. Whenever in these groups it does not occur, it is easy to account for its disappearance; in Nassa, for example, and in the Cephalopoda the form of cleavage is clearly correlated with the large amount of yolk present in the egg. There is often a strikingly close resemblance between the cell lineages that have been worked out in the two phyla, but too great stress should not be laid on this point, since the resemblances between the cleavage patterns may be taken to indicate a similarity of physical and mechanical conditions in the egg, rather than of any close phylogenetic relationship. However that may be, Carazzi's work on the cell lineage of Aplysia only adds another to the already long list of remarkable parallels in this respect between the two groups.

Our own work is more directly concerned with the origin and fate of the mesoderm in Annelids and Molluscs. In all Annelids in which the cell lineage has been investigated, the cell known as 4*d* gives rise to the most important part of the mesoderm, and sometimes to all the mesoderm, as in Aplysia. In some other forms the so-called larval mesoderm derived from the ectoderm contributes to a greater or less extent to the structure of the larva. In Annelids the subsequent history of the mesoderm is well known; and it is interesting to find that in many Molluscs well-developed mesodermal bands are found, in all ways comparable to those in Annelids. As might be expected, the best examples of mesodermal bands occur in the more primitive groups of Molluscs. Kowalevsky has described them in Chiton polii, and Heath in Ischnochiton. Among the Solenogastres also we find in Dondersia and Proneomenia that the bands are unusually distinct, and Patten figured them clearly in Patella. But in the more modified groups of the Mollusca one could hardly speak of mesoderm bands except on the analogy of the less specialised forms. This is the case in Aplysia, where the bands are never clearly defined and soon break up into scattered cells.

Turning to the development of the cœlom, it is here that we find the first essential difference between Annelids and

Molluscs. For in the latter phylum there is never any trace of segmentation, whereas in the former, as is well known, the bands become split up into blocks, in each of which a cœlomic cavity is formed. A further difference is the reduction of the cœlom in Molluscs, but this is by no means so great as is usually supposed. Though the evidence is as yet scanty owing to the small amount of work that has been done on the later stages on development, nevertheless there is reason to believe that the cœlom is, at a late period in the metamorphosis, of considerable size, and that even in the adults of some of the more primitive groups it remains large. Kowalevsky long ago described the development of the cœlom in Chiton polii, and some of his figures, which show the cœlom surrounding the gut, would pass well for a transverse section of an Annelid larva at a late stage. It is very probable that a similarly extensive development of the cœlom will be found in the Solenogastres, where, as we have seen, the mesoderm bands are of considerable size. Among the Aspidobranchia, as the most primitive of the Gastropoda, we might expect a larger body cavity than in the more specialised forms, if one regards the extensive cœlom as a primitive factor preserved from an Annelid ancestor; but unfortunately nothing is known about the later larval stages. In a recent account of the structure of the Neritidæ, Bourne, however, has lately described a very large cœlom, more extensive than has been described in any other gastropod. To find a parallel to it we must refer, he says, to the Cephalopoda. There are a number of descriptions of the development of the Pectinibranchia, in which the cœlom is extensive; all the authors who have worked on Paludina agree upon this. Other cases are Vermetus, where Salensky describes a somatopleur and a splanchnopleur, though unfortunately he gives no figures; and Bithynia, described and figure by Erlanger. Among Pulmonates and Lamellibranchs, as one might expect in such specialised forms, we find no evidence of the existence of a large cœlom, this structure being reduced in every case to a small sac-like pericardium and a reno-pericardial duct. These examples will, however, serve to show that a well-developed cœlom is of frequent occurrence in the Mollusca, and that it is probable that when the later stages in other Molluscan groups have been more thoroughly examined, a large cœlom like that which we have described in Aplysia will be found to be a normal feature in the organisation of the Molluscan larvæ. But it is to be observed that the cœlom in Aplysia is developed at a stage when in both Annelids and Molluscs the mesoderm bands are still intact and a cavity has not yet been developed.

In very few forms among the Mollusca has the development of the cœlom been traced from the segmentation period onwards. Among the forms which have been worked out, Aplysia and Physa follow what we may call the normal Annelid type, that is to say, the mesoderm, all of it in Aplysia, and the greater part of it in Physa, is developed from $4d$, and from it the cœlom arises. In the others there is a departure from this type of development; there are Dreissensia, Limax, and Cyclas, which have been described by Meisenheimer, and Paludina, according to Otto and Tönniger. In the first three cases the cell lineage is known, and $4d$ develops in the usual way and gives rise to bands, which split up and form mesenchymatous tissue, and thus seems to correspond to the larval mesoderm described by Lillie in the Unionidæ, where it arises from $2a^2$, but is believed to give rise to the adductor muscle. The cœlom is stated to arise, not from the descendants of $4d$, but from cells which proliferate from the "ectoderm" at a comparatively late stage when segmentation is complete. The same is said to be the case with Paludina; the cell lineage is not known in this form, but it is distinctly stated that there are no pole mesoderm cells.

Such a marked departure from the typical mode of development was hardly to be looked for; it is to be noticed that it occurs in widely separated members of the phylum, and further, that there are no peculiar bionomic conditions common to them. So far as our knowledge goes, it would seem to be an alternative mode of development, which may occur anywhere in the Mollusca. At first sight it might seem to involve serious difficulties as to the homology of the organs formed by these very different processes, but it would be clearly absurd to argue from this want of resemblance in the method by which the cells giving rise to the cœlom are segregated during development that the cœlom and its derivatives are therefore not homologous throughout the Mollusca. Evidently the heart, pericardium, and kidneys of adult molluscs are all homologous. It might thus seem that the evidence of embryology was worthless in this case; but these two modes of development are not so different as might seem at first sight. For, although a superficial examination of Molluscan cell lineages leads one to expect that mesodermal structures are always formed from the descendants of 4*d* at a parallel stage in development, closer inspection shows that this is by no means invariably the case. The period at which the mesoderm becomes segregated from the other embryonic elements varies considerably; it takes place in Planorbis marginatus when there are only twenty-four cells present; in Planorbis trivolvis when there are forty-nine; and in Trochus magnus when there are 145. Statements have also been made that in Tethys and Teredo 4*d* does not give rise to mesoderm at all. Differences in the mode of segregation are thus to be found in closely allied genera, and we cannot lay down any hard and fast rule to govern developmental processes even in the same phylum. All that we are justified in saying in the present state of our knowledge is that there are certain definite organ-forming substances present in the egg before segmentation begins which are homologous throughout the group. As this process takes place these may be separated into definite cells or groups of cells, from which the corresponding organs, or complex of organs, are subsequently developed; but this is by no means necessarily the case. The factors for the formation of certain organs, as, for example, the cœlom and related structures above mentioned, instead of being aggregated at an early stage into a single cell, may be localised in many different cells with a totally different destiny, and only at a comparatively late stage become finally segregated out, in the present case by proliferation.

That the way in which organ-forming substances present in the egg are finally separated from one another is quite immaterial in affecting the homologies of the organs to which they give rise is very clearly demonstrated by certain experimental work, as, for example, that of Wilson on the egg of Nereis. In this case the cleavage pattern was totally changed by subjecting the egg to pressure, and yet the larva produced was normal.

These facts, taken together with what we know of the movements of the cytoplasm before and during segmentation in Cynthia, Dentalium, Cerebratulus, etc., show that the organ-forming substances often shift their position, and are segregated at different periods. Meisenheimer's results have demonstrated a remarkable instance of this, but provide no evidence concerning the homologies of the organs.

Before we discuss the larval excretory organs in Aplysia, we may briefly describe the types found among Molluscs.

I. Flame-Cells:

α. The flame is borne by one cell only; the duct is intracellular.

(1) Organ consists of two cells: Lamellibranchs.

(2) Organ consists of four cells: Fresh-water Pulmonates and Basommatophora.

β. The flames are borne by more than one cell; the duct is inter-cellular: Terrestrial Pulmonates; Stylommatophora and Paludina.

II. Ectoderm Cells which enlarge, become vacuolated and project from the surface.

α. Position variable, but near the base of the velum: Marine Prosobranchs.

β. Position definite, slightly anterior to the anus: Opisthobranchs.

III. Nephrocysts (primitive kidneys). A single cell some distance beneath the surface, and without a duct: Opisthobranchs.

IV. Secondary kidneys. Several large vacuolated ectoderm cells opening to the exterior by a short duct: Opisthobranchs.

The origin of Type I has been differently described by several investigators, and though there seems to be much evidence of its arising from the ectoderm, yet a mesodermal origin has been ascribed to it by Erlanger in Paludina, Stauffacher in Cyclas, and Rabl and Holmes in Planorbis. If the ectodermal origin of these larval excretory organs should be proved beyond dispute, we should fairly be able to compare them with the Annelid nephridia. Nevertheless, if these organs are taken as representing ancestral nephridia, and thus indicating a relationship between the Annelid and Molluscan phyla, it is remarkable that they have never been found in the primitive groups of the latter, the Amphineura and the Aspidobranchia, and yet are present in the highly specialised Pulmonates. The second type of larval excretory organ is of no special significance. It has obviously been developed to meet some special need during the early stages of ontogeny. Type III may possibly be of a similar nature to the preceding, but it certainly stands apart from the others, and until we know whether it is ectodermal or mesodermal, as has been asserted by Mazzarelli, it is impossible to compare it with any other form of excretory organ. The fourth type of kidney, which we have called the secondary kidney of Opisthobranchs, offers some difficult problems both as regards its homology and its ultimate destiny. The position it occupies is very similar to that of the definitive kidney in the adult. Mazzarelli, when he originally described it as arising from the mesoderm, believed it to be the rudiment of that organ. This idea he has abandoned in his later work on the free-swimming larvæ of Opisthobranchs, and is inclined to believe that the organ disappears in the metamorphosis, and is in no way connected with the adult organ. He produces no evidence for this view, though, as will appear later, we think it has much justification. Casteel thinks it probable that the secondary kidney persists through the metamorphosis and becomes the kidney of the adult in spite of its ectodermal origin; he supports his view by referring to Meisenheimer's account of the "ectodermal" origin of the common rudiment of heart, kidney, pericardium, and gonad in Dreissensia and other forms. Heymons considered the kidney to be merely a larval organ; he further compared it to the external ectodermal kidneys of Prosobranchs, our Type II. This suggested homology seems to us very far fetched. To begin with, these Prosobranch kidneys are variable in position in the same species, while the Opisthobranch kidneys are derived from almost the same cell in the three forms, Aplysia, Fiona, and Umbrella. Further, the Prosobranch kidney is an external protruding organ, while the Opisthobranch kidney sinks well below the surface epithelium.

Our own conclusion is that the secondary kidney of Opisthobranchs cannot be homologised with any of the other various molluscan kidneys. We have already given our reason for believing that it cannot be homologised with our Type II. The fundamental difference between it and our Type I is that cilia are absent. It is possible that in such advanced forms as the Opisthobranchs the cilia might have been lost and the nephridium reduced to some such condition as that which we find in the secondary kidney. But the posterior position of the organ makes it unlikely that it has anything to do with the Annelid nephridium, the representative of which in Molluscs is always found close up under the velum. In the embryo of terrestrial Pulmonates, also, the nephridium is preserved, although they are more modified than the Opisthobranchs.

The position of the secondary kidney suggests at first sight that it is the rudiment of the definitive kidney. But in all those cases in which the origin of the definitive kidney is known for certain, and in which it has been traced from the embryo to the adult, it has been found to arise as an evagination from the cœlomic epithelium, which joins an ectodermal invagination and so reaches the exterior. A communication between the cœlom and the kidney is present from the earliest stage in the formation of the latter, and persists as the reno-pericardial aperture in the adult of all Molluscs with the exception of Nautilus. Now in Aplysia there is never any connection between the cœlom and the secondary kidney; there is no reno-pericardial duct in connection with it; the two organs, both in origin and later development, are perfectly distinct. We consider it therefore probable that the secondary kidney of Aplysia is a larval organ which degenerates and disappears during the metamorphosis, and that the definitive kidney arises as an evagination of the cœlomic epithelium as it does in Physa.

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[1] For a complete bibliography of the whole subject the reader is referred to Mazzarelli's monograph (**14**) for list of literature up to 1892, and to Carazzi's paper (**5**) for more recent work up to 1906.

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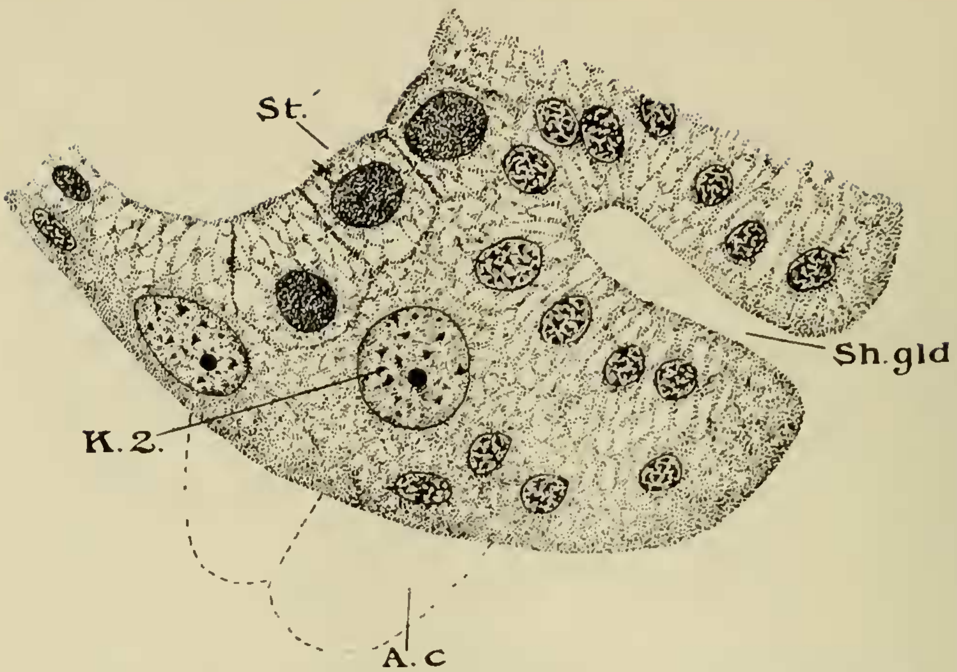
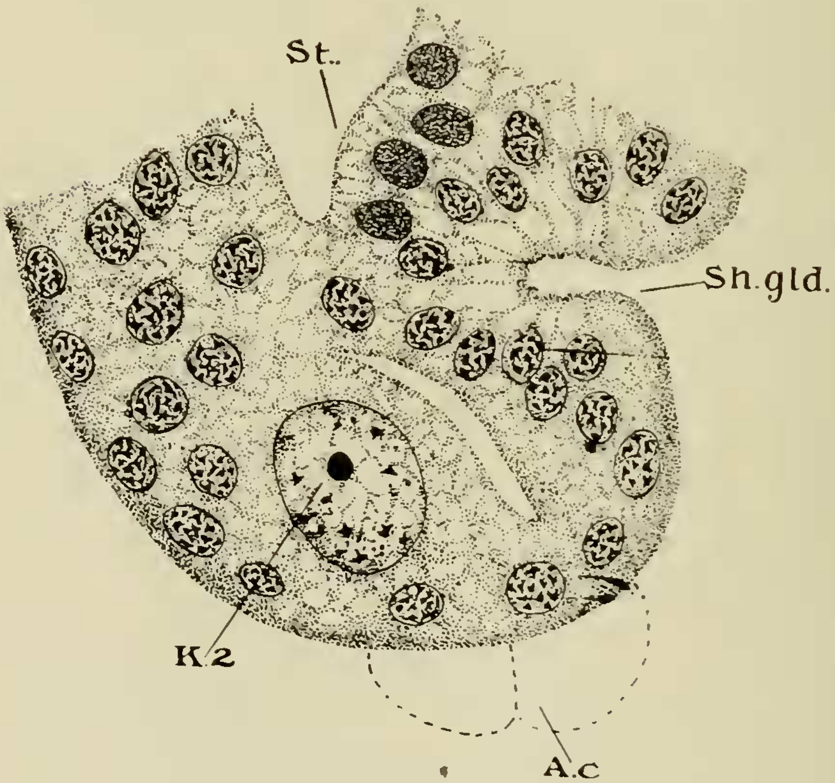
Text-fig. 15.[1]



Section through the right posterior region of an embryo in the same stage as figs. 1 and 2 of Pl. 22, showing the ectoderm cells, K. 2, which will form the secondary kidney, still lying on the surface.

Text-fig. 16.



Section through the same region of an embryo a few hours older.

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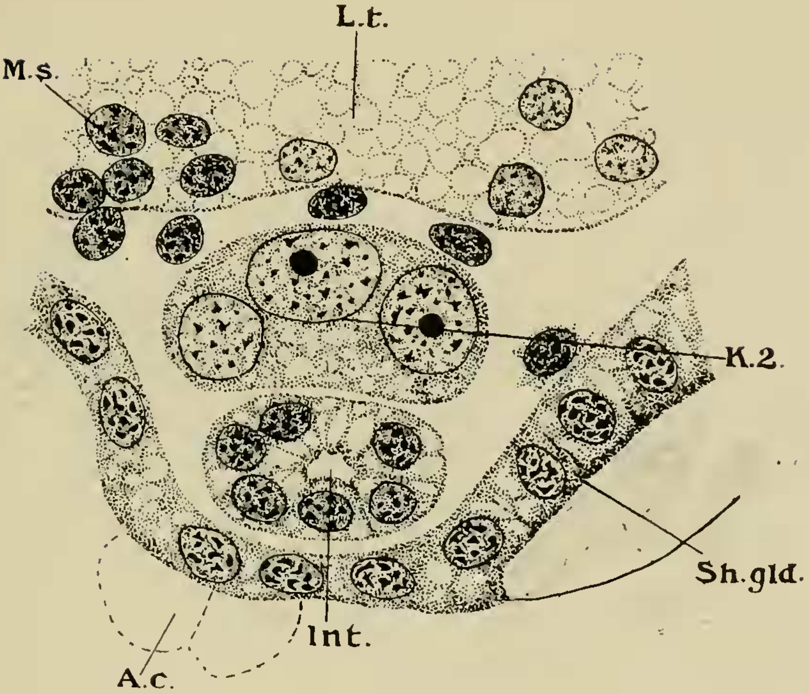
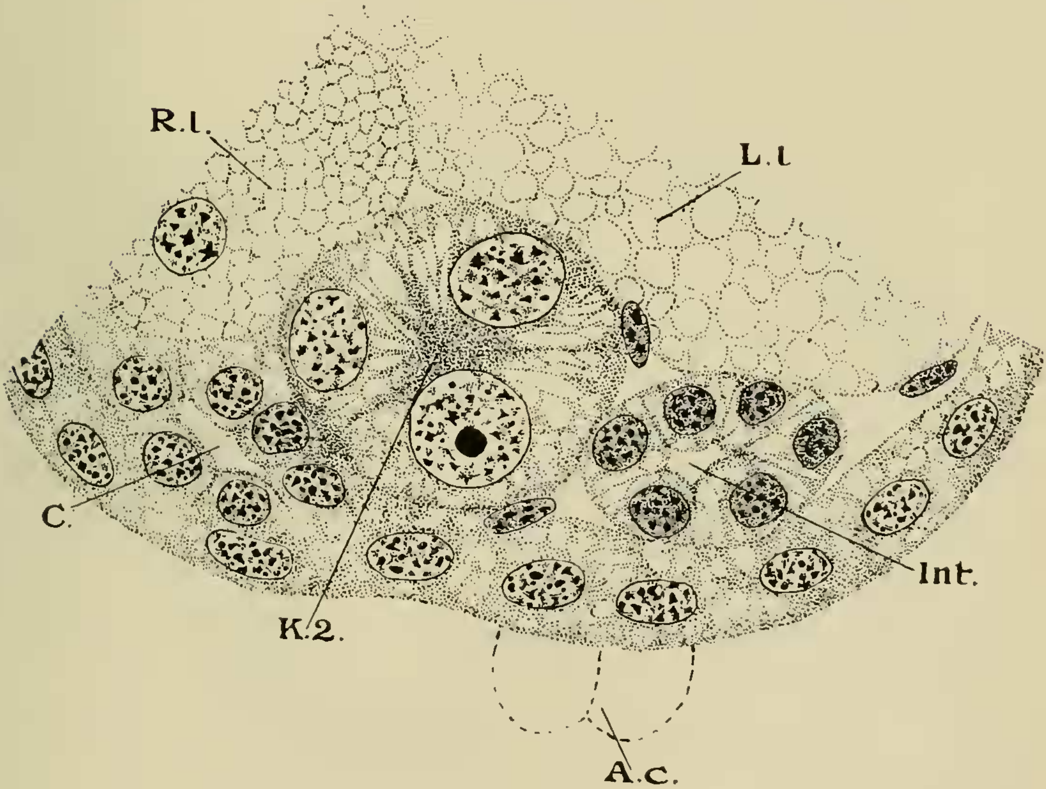
[1] Text-figs. 15–20 are drawn from sections 3 μ thick, with an Abbe camera with $\frac{1}{12}$ oil-immersion and a compensating eye-piece. Zeiss, No. 8, except Text-fig. 20, which is much less highly magnified. N.B.—For signification of the lettering of figs. 15 to 20 see the explanation of the same letters in Pl. 22, given on p. 539.

TEXT-FIG. 17.



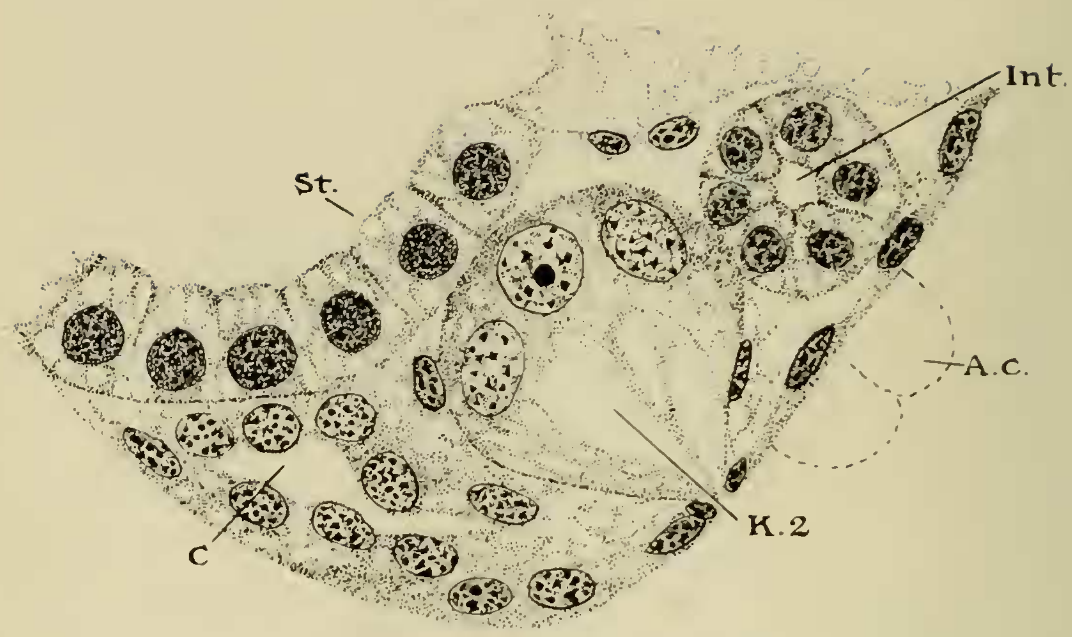
Section through the same region of an embryo in the same stage as in fig. 4 of Pl. 22, showing the first signs of the accumulation of mesoderm cells in which the cœlom is formed at a later stage.

TEXT-FIG. 18.



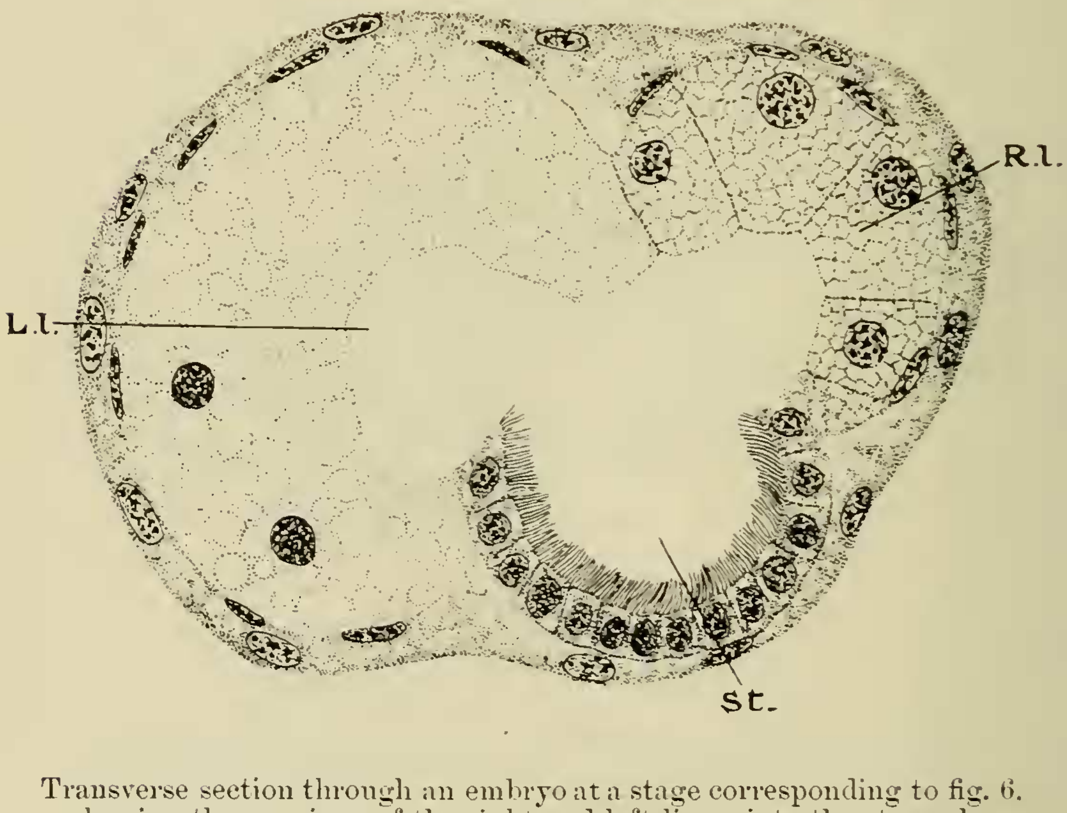
Section through approximately the same region of a slightly older embryo. The cœlom is now a definite cavity bounded by the mesoderm cells. The secondary kidney is here shown cut across its long axis.

Text-fig. 19.



Section through the same region of an older embryo, but taken almost at right angles to the section drawn in Text-fig. 18. The cœlom has increased in size and is beginning to extend dorsally between the body-wall and the stomach. The secondary kidney is cut longitudinally to show its vacuolated structure and the duct opening into the mantle-cavity.

Text-fig. 20.



Transverse section through an embryo at a stage corresponding to fig. 6. showing the openings of the right and left livers into the stomach.

EXPLANATION OF PLATE 22,

Illustrating Mr. A. M. Carr Saunders and Miss Margaret Poole's paper on "The Development of Aplysia punctata."

Explanation of Abbreviations in Figures.

A. Anus. *A.c.* Anal cells. *C.* Cœlom. *C.c.* Cerebral commissure. *C.d.e.* Dorsal extension of cœlom. *C.g.* Cerebral ganglion. *C.v.e.l.* Ventral extension of cœlom on left side. *C.v.e.r.* Ventral extension of cœlom on right side. *F.* Foot. *Int.* Intestine. *K.*1. Primitive kidney. *K.*2. Secondary kidney. *L.l.* Left liver. *M.* Mouth. *M.c.* Mantle cavity. *Ms.* Mesoderm. *Œs.* Œsophagus. *O.* Otolith. *Ot.* Otocysts. *P.g.* Pedal ganglion. *R.l.* Right liver. *Sh.gld.* Shell-gland. *St.* Stomach. *V.* Velum.

N.B.—This explanation of lettering applies to Text-figs. 15 to 20 (pp. 536–538), as well as to the figures on Pl. 22.

Fig. 1.—Embryo at the stage immediately before the beginning of rotation, seen from the right side.

Fig. 2.—Same embryo seen in diagrammatic optical section; the ectoderm is represented as peeled off from the right half of the embryo and thus seen in section.

Fig. 3.—Slightly older embryo represented as in fig. 2.

Fig. 4.—Slightly diagrammatic view of an embryo about twenty-four hours older than that shown in fig. 3, seen from the right side.

Fig. 5.—Similar view of older embryo, showing first appearance of the cœlom (coloured red).

Fig. 6.—Similar view of still later stage. The embryo has now assumed its characteristic veliger form.

Fig. 7.—Similar view of an embryo a few hours before its emergence from the capsule.

Fig 8.—Similar view of a free-swimming larva.

Fig. 9.—Free-swimming larva seen from the dorsal surface.